Rudolphiella szidati sp. n. (Proteocephalidea: Monticelliidae, Rudolphiellinae) parasite of Luciopimelodus pati (Valenciennes, 1840) (Pisces: Pimelodidae) from Argentina with new observations on Rudolphiella lobosa (Riggenbach, 1895)

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Rudolphiella szidati sp. n. (Proteocephalidea: Monticelliidae, Rudolphiellinae) parasite of Luciopimelodus pati (Valenciennes, 1840) (Pisces: Pimelodidae) from Argentina with new observations on Rudolphiella lobosa (Riggenbach, 1895). - Rudolphiella szidati sp. n. (Cestoda, Monticelliidae, Rudolphiellinae) is described from the anterior intestine of the pimelodid fish, Luciopimelodus pati caught in Paraná river and de la Plata river (Argentina). Additional informations to the description of the type material of Rudolphiella lobosa (type species of the genus) is given. R. lobosa was not found in L. pati (as reported originally by Riggenbach, 1895) and has never been found since its original description. The new species differs from other species of Rudolphiella by its larger scolex, a higher number of testes and by its distinct egg shape. A diagnosis of *Rudolphiella* is given. The following taxonomical actions are introduced: Rudolphiella piracatinga (Woodland, 1935) comb. nov. for Monticellia piracatinga Woodland, 1935; Monticellia rugata Rego, 1975 syn. nov. of Rudolphiella piracatinga (Woodland, 1935). The presence of glandular cells within the scolex's apex is observed in all known Rudolphiella species i.e. R. lobosa, R. myoides, R. szidati, R. piracatinga, R. piranabu, Rudolphiella, the sole genus within Rudolphiellinae, is widespread in a monophyletic group of hosts (Calophysus-group sensu de Pinna, 1998) suggesting likely coevolution.

Key-words: Proteocephalidea – Monticelliidae – *Rudolphiella szidati* sp. n. – *Rudolphiella lobosa* – *Luciopimelodus pati* – coevolution – Argentina.

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INTRODUCTION

Riggenbach (1895) described *Corallobothrimn lobosmu* parasite of *Luciopimelodns pati* (Valenciennes, 1840) from Paraguay River, Paraguay. Later, Fuhrmann (1916) redescribed Riggenbach's material adding information of proglottides' transverse sections, and erected a new genus *Rndolphiella* Fuhrmann, 1916 to allocate *Corallobothrimn lobosmu* Riggenbach, 1895. This species has never been found since its original description.

Woodland (1934) erected the genus *Amphilaphorchis* and described two species *Amphilaphorchis piranabn* Woodland, 1934 and *A. myoides* Woodland, 1934, both parasites of *Pinirampns pirinampn* (Spix, 1829). A year later Woodland (1935), concluded that genus *Amphilaphorchis* Woodland, 1934 is a synonym of *Rndolphiella* and erected a new subfamily, the Rudolphiellinae.

The examination of *Lnciopimelodns pati* (Valenciennes, 1840) from Paraná river and de la Plata river. Argentina, the type host of *R. lobosa* (Riggenbach, 1895) revealed the presence of a new species of *Rudolphiella* described herein. Since the original description of *Rudolphiella lobosa* (Riggenbach, 1895) did not contain data on some useful morphological structures, the type material of this species is restudied.

MATERIAL AND METHODS

Sixty-two specimens of *Luciopimelodus pati* (Valenciennes, 1840) were examined for helminths. Forty intestines were placed in lukewarm water in order to relax the worms and then fixed in AFA, while others were dissected and fixed directly in hot 4% formaldehyde solution and were subsequently stored in ethanol 75 % V/V. Entire tapeworms were stained with Langeron's alcoholic chlorhydric carmine (Langeron, 1949), differenciated in acid ethanol, dehydrated in ethanol, cleared in beechwood creosote or in eugenol, and mounted in Canada balsam. Thick transverse hand-cutting sections of the proglottides were stained following equivalent procedure. Two scolices and pieces of strobilas were embedded in paraffin, transversely sectioned at 12-15 µm, stained with Weigert's hematoxylin and counterstained with 1% eosin B and mounted in Canada balsam. Eggs were mounted in distillated water for drawing. Two scolices were prepared for scanning electron microscopy (SEM), they were dehydrated through a gradual series of ethanol, then put in amyl acetate, critical point dried and sputtered with gold and photographed with a Zeiss DSM 940 A SEM.

Rudolphiella szidati sp. n. type material was deposited at the Helminthological Collection of the "Museo Argentino de Ciencias Naturales Bernardino Rivadavia". Buenos Aires, Argentina (MACN), and at the Natural History Museum, Geneva, Switzerland (MHNG). We also studied syntypes of *R. piranabn* (Woodland, 1934) BMNH 1964.12.15.101.107 and syntypes of *R. myoides* (Woodland, 1934) BMNH 1964.12.15.108.110, (Amaz 74.3), both parasites from *Pinirampus pirinampu* (Spix. 1829); syntypes of *R. piracatinga* (Woodland, 1935) BMNH 1964.12.15.206-208, (Amaz 40) from *Calophysns macropterns* (Lichtenstein, 1819) from the British Museum of Natural History. London (BMNH). Amaz = field numbers of Woodland's material.

All measurements are given in micrometres, unless otherwise stated, with the range followed by the mean (m) and the number of measurements (n) in parentheses. MT = type material. Illustrations were made with the aid of a camera lucida.

RESULTS

Rudolphiella Fuhrmann, 1916

Syn. Amphilaphorchis Woodland, 1934

Diagnosis: Proteocephalidea, Monticelliidae, Rudolphiellinae. Worms of small or medium size; wrinkle collar-like metascolex; suckers uniloculate with developed internal circular musculature in their distal margin; apical glandular cells arranged in a cross situated between the suckers and the apical tegument; internal longitudinal musculature developed; testes cortical in one layer; vagina posterior or anterior to cirrus pouch, when anterior ventrally overlapping the cirrus pouch; genital pores irregularly alternating: ovary medullar with outgrowths in cortex, strongly lobulate; vitelline follicles cortical, ventral, with a tendency of posterior concentration; uterine primordium and lateral branches medullar; eggs with elongated shell and with embryophores bearing two elongated polar projections; all species parasites of catfishes of the family Pimelodidae. Type species: Rudolphiella lobosa (Riggenbach, 1895)

Rudolphiella szidati sp. n.

Figs 1, 3-5, 8, 11

Host: Luciopimelodus pati (Valenciennes, 1840) (= Pimelodus pati), common name: patí.

Material studied: Argentina, Provincia de Corrientes, Ciudad de Corrientes, Puerto Italia (Paraná river), holotype MHNG: 26251 INVE, 30.07.1997, paratypes MHNG: 26252 - 26256 INVE, 30.07. to 01.08.1997; Argentina, Provincia de Chaco, Pte General Belgrano (Paraná river) (27° 27′S, 58° 50′W), 8 paratypes MACN: 392/1-8; Argentina, Provincia de Buenos Aires, Puerto de Buenos Aires (de la Plata river) (34° 37′S, 58° 22′W), MACN 392/9-10, 2 paratypes, 06.06.1995; other material: Argentina, Provincia de Corrientes, Ciudad de Corrientes, Puerto Italia (Paraná river), 24667 - 24674, 27235 INVE, 30-31.07.1997 and MACN 392/11-15.

Site of infection: anterior portion of intestine and rarely in first portion of medium intestine.

Prevalence: 100%, 62 fishes examined.

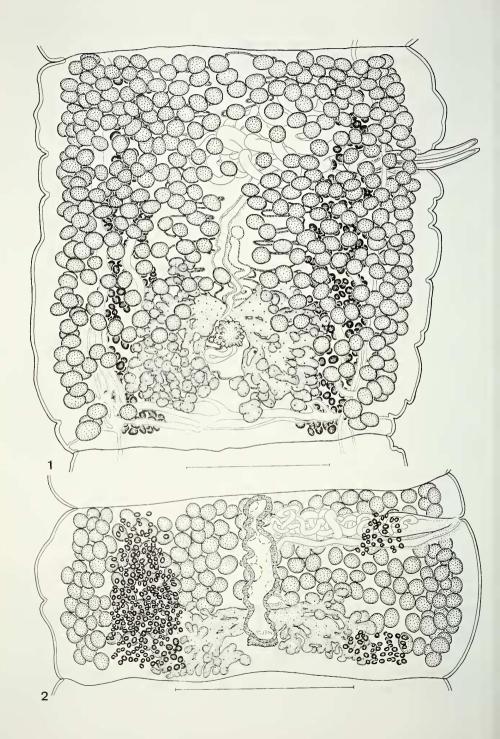
Intensity: 2-52; m = 20

Etymology: the new species honours Prof. Lothar Szidat (Buenos Aires).

DESCRIPTION (based on 30 specimens and measurements on 8 specimens):

Monticelliidae, Rudolphielliinae. Testes cortical, situated dorsally and surrounding laterally and ventrally the longitudinal muscular bundles, reaching but not overlapping the vitelline follicles (Fig. 4); ovary medullar with projections into the cortical parenchyma (Fig. 5); uterus medullar (uterine stem and lateral branches). Vitelline follicles cortical in 2 ventral bands (Figs 1, 4-5).

Medium size worms, flattened dorso-ventrally, total length 16-27 mm (m = 22, n = 8). Strobila with wrinkles and furrows, acraspedote, comprising about 22-37 (m = 28, n = 8) proglottides, fast maturation (10-18 immature proglottides, 4-6 mature proglottides and 6-10 gravid proglottides).



Wrinkle collar-like metascolex (in the sense of de Chambrier & Paulino, 1997) (Fig. 11), encircling the anterior part of the scolex, 1735-2970 in diameter. Metascolex with apical elongated bottle-shape glandular cells with granular inclusions, arranged in a cross situated between the suckers and the apical tegument (Fig. 3a): 4 slightly oval uniloculate suckers. 395-595 (m = 490, n = 19) long and 345-560 (m = 415. n = 19) wide; presence of internal circular musculature on distal margin of suckers (Fig. 3b); suckers usually hidden by the collar-like metascolex. The suckers are visible when the scolex is fixed outside the gut.

Proliferation zone 990-2970 (m = 2005, n = 8) long, 625-1050 (m = 845, n = 8) wide. Immature proglottides 130-530 (m = 310, n = 26) long, 695-1485 (m = 1080, n = 26) wide; mature proglottides 380-1110 (m = 610, n = 34) long, 925-1780 (m = 1330, n = 34) wide. Gravid proglottides 495-1500 (m = 965, n = 37) long, 775-1895 (m = 1425, n = 37) wide. Last proglottis 2145 long, 990 wide.

Internal longitudinal musculature developed, forming a thick bundle of thin muscular fibres (Figs 4-5). Osmoregulatory canals thick-walled, with anastomoses in the last third of proglottis. Ventral canals interconnected posteriorly to ovary (Fig. 1). Ventral canals 20-45 in diameter and dorsal canals 8-30 in diameter. A thin lateral ventral secondary duct of ventral osmoregulatory canal opens on each posterior side of the proglottis, forming a vesicle before ending in the tegument. This structure was also observed in *R. lobosa* by Riggenbach (1896).

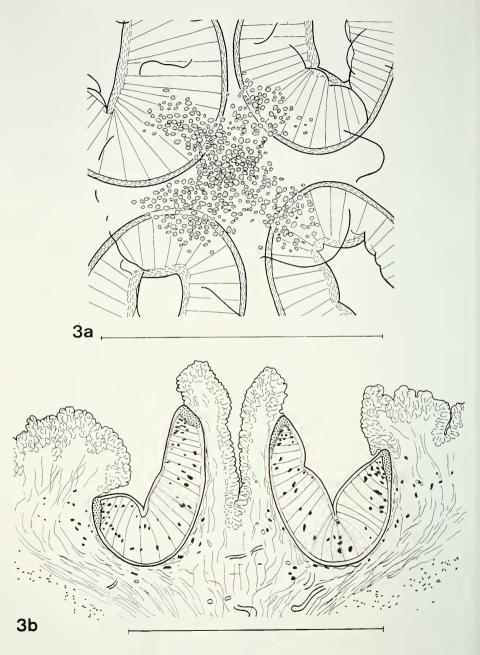
Testes spherical, 50-70 in diameter, 289-365 (m = 325, n = 6), one-layered, in a compact single field, located in the cortical parenchyma, dorsal, lateral and ventral in part, not interrupted at cirrus pouch level; overlapping the anterior margin of the ovary and laterally reaching the posterior end of the proglottis (Fig. 1).

Genital pores alternating irregularly, in 20-46% (m = 30%, n = 34) anterior of proglottis length. Ejaculatory duct coiled. Cirrus pouch thin-walled, 230-330 (m = 270, n = 34) long, 60-110 (m = 85. n = 34) wide. Cirrus pouch length occupying 15-32% (m = 23%, n = 34) of proglottis width. Vas deferens bulky and very coiled, sometimes reaching body midline, usually reaching anterior margin of mature proglottis, occupying approximately one third of mature proglottis length (Fig. 1).

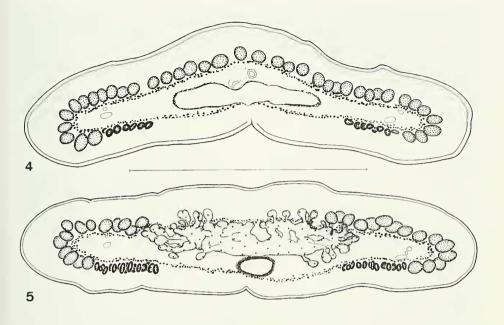
Vagina thick- walled, forming 1-2 loops near the ovary. Vagina posterior (77%) or anterior (23%) to cirrus pouch; when the vaginal duct is anterior, ventrally overlapping cirrus pouch, not surrounding the coiled vas deferens as is common. Ovary medullar, bilobed, strongly lobulate with dorsal and ventral outgrowths, only dorsal projections reach the cortex. Ovary occupying 43-68% (m = 53%, n = 34) of proglottis width (Fig. 1).

Figs 1-2

^{1.} Rudolphiella szidati sp. n., holotype 26251 INVE, pregravide proglottis, dorsal view. 2. Rudolphiella lobosa (Riggenbach, 1896), syntype-material, 43/43, pregravide proglottis, ventral view (coll. Zoological Institute, Neuchâtel). The vitelline follicles on the right side are not entirely represented. Scale-bar, 500 μm.



Figs 3a-b. *Rudolphiella szidati* sp. n. 3a. 24673 INVE. apical view of scolex, detail of the apical portion showing the granular cells in a cross shape situated below the tegument. 3b. 27235 INVE, parasagittal section of scolex showing the internal circular musculature in their distal margin. Scale-bar, $500 \, \mu m$.



Figs 4-5

Rudolphiella szidati sp. n., paratype, 26253 INVE, transverses sections of a pregravide proglottis. 4. Sections at ovary level; 5. Sections at posterior part of proglottis. Scale-bar, 1000 μm.

Vitelline follicles cortical in 2 ventral bands, sometimes reaching anterior and posterior margin of proglottis, with a tendency to a posterior concentration of follicles, uninterrupted by cirrus pouch and vagina (Figs 1, 4-5).

Uterine primordium medullar originating from a cylindrical mass of chromophil cells. Lumen appearing in first mature proglottides. Medullar uterine branches up to 70% (m = 34%, n = 37) of gravid proglottis width, with 9-17 lateral branches opposite to cirrus pouch and 7-16 on cirrus pouch side.

Eggs with elongated polar projections aproximately of equal size (Fig. 8); thin, hyaline and spindle-drop shaped external shell. Outer envelope 128-167 (m = 140, n = 12) long. 20-23 (m = 21, n = 12) wide. Inner envelope consisting in bilayered embryophore, with nucleate envelope 16-21 (m = 20, n = 12) long, 10-16 (m = 14, n = 12) wide and external layer 40-50 (m = 45, n = 12) long, 18-21 (m = 20, n = 12). Oncosphere slightly oval, 10-13 (m = 11, n = 12) long, 7-13 (m = 10, n = 12) wide; oncosphere hooks, 3-6 (m = 4, n = 17); According to Swiderski (1994), we interprete the described structures as: 1. Shell; 2. Outer envelope; 3. Inner envelope consisting in bilayered embryophore with external layer much bigger than nucleate envelope; 4. Oncospherical membrane rarely visible: 5. Oncosphere.

Rudolphiella lobosa (Riggenbach, 1895) Fuhrmann, 1916

Fig. 2

syn. Corallobothrium lobosum Riggenbach, 1895; Ephedrocephalus lobosum (Riggenbach, 1895) Mola, 1906.

Host (according to Riggenbach, 1895): *Pimelodus pati* (= *Luciopimelodus pati* (Valenciennes, 1840), common name: patí.

Material examined: Paraguay. Paraguay river, type-material, slides 43/43-44 (Collection of the Institute of Zoology, Neuchâtel, deposited in the MHNG), collected by Ternetz. 01-02.1894.

Site of infection: intestine.

DESCRIPTION:

Strobila acraspedote, with wrinkles and furrows; worms of medium size 17-22 mm long, with fast maturation. Scolex with apical glandular cells arranged in a cross, situated between the suckers and the apical tegument, presence of a well developed metascolex, 1025-1350 wide. Suckers uniloculate 205-245 in diameter, with free distal muscular sphincter. Proliferation zone about 800 long. Longitudinal muscular fibers developed all around the proglottis.

Testes in one layer in transverse sections, in a single field, sphaerical 50-65 in diameter, 194-219 (m = 206; n = 3) (53 in original figure of Riggenbach (1896), 150-200 according to Riggenbach (1896), 220 according to Fuhrmann (1916)) in number. Testes in cortical parenchyma, dorsal, lateral and ventral in part, not interrupted at cirrus pouch level; overlapping anterior margin of ovary and laterally reaching posterior end of the proglottis (Fig. 2).

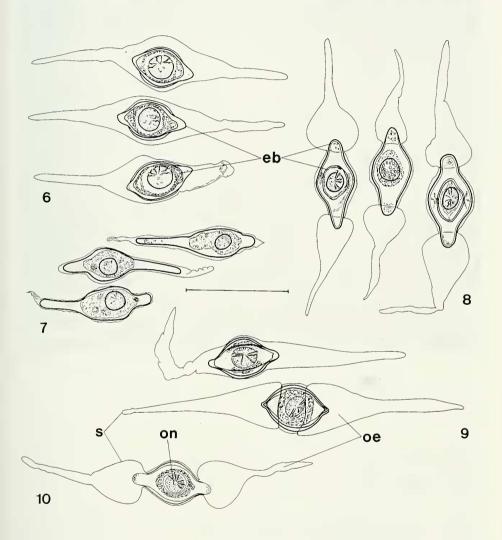
Genital pores alternating irregularly, in 21-32% (n = 6) of proglottis length. Cirrus pouch thin-walled, 170-205 long. Cirrus pouch length occupying 16-22% (n = 4) of proglottis width. Vas deferens not reaching body midline in mature proglottis.

Vagina posterior (53%) or anterior (47%) to cirrus pouch, with inconspicuous terminal muscular sphincter. Ovary medullar, bilobed, strongly lobed and bearing dorsal projections penetrating the cortex: occupying 60-72% of proglottis width. Vitelline follicles distributed cortically and ventrally, in two lateral bands, sometimes reaching anterior and posterior margin of proglottis, with a tendency of posterior concentration, uninterrupted by cirrus pouch and vagina.

Uterine primordium like a medullar cylindrical mass of chromophil cells. Lumen appearing from the first mature proglottides until formation of eggs. Uterine branches occupying up to 56% of proglottis width, with 8-12 lateral branches on each side, 1or 2 layered. Eggs with two polar projections.

Remarks: The new species belongs to Rudolphiella Fuhrmann, 1916 based on the cortical distribution of the testes and vitelline follicles, on the medullar position of uterus, as well as on the medullar and partially cortical location of the ovary (Rego, 1994). Four species of Rudolphiella are present: R. lobosa (Riggenbach, 1895) recorded in Luciopimelodus pati: R. myoides (Woodland, 1934) and R. piranabu (Woodland, 1934), both parasites of Pinirampus pirinampu; Rudolphiella piracatinga (Woodland, 1935), parasite of Calophysus macropterus. Rudolphiella cf. lobosa de Chambrier & Vaucher, 1999 parasite of Megalonema platanum is also considered.

Rudolphiella szidati sp. n. differs from all described species in the genus by the size of metascolex and by the testes number (Table 1). Furthermore, in R. szidati, (with R. lobosa and R. cf. lobosa) testes laterally reaching the posterior end of proglottis, while in R. myoides and R. piranabu the number of testes decreases and they do not



Figs 6-10

Rudolphiella spp., Eggs drawn in distilled water. 6. Rudolphiella piranabu (Woodland, 1934), 25129 INVE; 7. Rudolphiella myoides (Woodland, 1934), 24712 INVE; 8. Rudolphiella szidati sp. n., 24670 INVE; 9. Rudolphiella piracatinga (Woodland, 1935), 19650 INVE; 10. Rudolphiella cf. lobosa, 22352 INVE (de Chambrier & Vaucher, 1999, fig. 95). Several eggs are not totally ripe. Abbreviations: eb = bilayered embryophore, oe = outer envelope, on = oncosphere, s = shell. Scale-bar, 50 µm

reach the posterior end of proglottis (de Chambrier & Vaucher, 1999; Pavanelli & Machado dos Santos, 1992; Riggenbach, 1895; Woodland, 1934, 1935).

Within the genus *Rndolphiella*, the eggs have a similar pattern with elongated poles, *R. szidati* (Fig. 8), *R. piracatinga* (Fig. 9), *R. cf. lobosa* (Fig. 10) and *R. piranabn* (Fig. 6) possess poles of similar size, but the shape is different in the 4 taxa; in *R. myoides*, one pole is shorter than the other (Fig. 7). The oncospheres of the 4 known species are similar in size (9-13), the outer envelope is difficult to compare among species based on uncertainties in the descriptions by different authors.

DISCUSSION

As we observed some more characters which are shared by all *Rudolphiella* species (i.e. presence of glandular cells within the apex, vagina anterior/posterior, shape of eggs), we gave above a new diagnosis of the genus.

Luciopimelodus pati is a common dweller fish of the Paraguay river, Bermejo river, Paraná river, Uruguay river, Carcarañá river and de la Plata river. The distribution of 3 Pimelodidae sharing the same common name (« patí »), Megalonema platanum (Günther, 1880), Pinirampus pirinampu (Spix, 1829) and Luciopimelodus pati (Valenciennes, 1840) is overlapping (Ringuelet et al., 1967). Since we were unable to find the species of parasites described by Riggenbach (1895) (neither R. lobosa nor Proteocephalus fossatus), from L. pati, we suspect that the host studied by Riggenbach was not Luciopimelodus pati, but possibly Megalonema platanum; this host was studied for cestodes by de Chambrier & Vaucher (1999) and it was parasited by Rudolphiella cf. lobosa.

Rego (1975) described Monticellia rugata from Calophysus macropterus in Amazonia. This cestode is a mixture of the two species Nomimoscolex piracatinga Woodland, 1935 and Monticellia piracatinga Woodland, 1935. The figures 44, 45, 47, 49 and 50 belong to Monticellia piracatinga and figures 46 and 48 belong to Nomimoscolex piracatinga. This latter species was transfered to the genus Monticellia under the name Monticellia amazonica by de Chambrier & Vaucher (1997). «Piracatinga» is the vernacular name given to Calophysus macropterus in Amazonia and to Luciopimelodus pati in high Paraná river in Brazil. Fowler (1951) and Ringuelet et al. (1967) placed L. pati into the Paraná bassin, but not in the Amazon bassin. We think that Woodland could be mistaken in attributing the name piracatinga to L. pati in Amazonia. Our recently collected material in Amazonia from Calophysus macropterus fits with the type material of the two species described by Woodland (M. piracatinga and N. piracatinga) and seems to confirm our hypothesis. Monticellia rugata Rego, 1975 is thus a junior synonym of Monticellia piracatinga. However, the study of type material of M. piracatinga Woodland, 1935 revealed that this species belongs to the genus Rudolphiella, as it is confirmed by the recent collected material from Calophysus macropterus in the Amazon and becomes Rudolphiella piracatinga (Woodland, 1935) comb. nov.

So far, numerous proteocephalideans possess unicelullar glands in the scolex (de Chambrier *et al.*, 1992, 1996; de Chambrier & Vaucher, 1997, 1999; Gil de Pertierra,

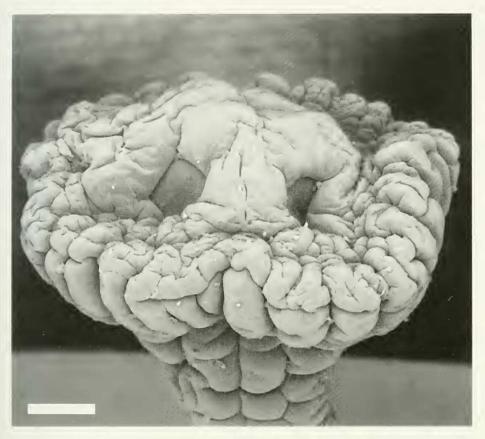


Fig. 11 Scolex, *Rudolphiella szidati* sp. n., 24669 INVE. Scale-bar, 200 µm.

1995: Gil de Pertierra & Viozzi, 1999; Scholz *et al.*, 1998, 1999: Stoitsova *et al.*, 1995). These glands are also present in all known species of *Rudolphiella* in high number and arranged in a cross, with granular inclusions. Zd´árská & Nebesárová (1999) give a detailed description of the unicellular glands situated under the apex of the scolex in *Proteocephalus macropterus*.

The presence of *R. myoides* and *R. piranabu* was confirmed in the host *Pinirampus pirinampu* from Amazon river by one of the authors (A. de Chambrier), and *R. piranabu* was confirmed by Pavanelli & Machado Dos Santos (1992) in the same host from Paraná river. Brazil.

Brooks (1984, 1995) already demonstrated, in phylogenetic analyses based on morphological characters, the monophyly of three *Rudolphiella* species (*R. lobosa*, *R. myoides* and *R piranabu*) based on the presence of elongated egg. However, his 1995 analysis showed some homoplasious characters and is consequently somewhat less

TABLE 1. Comparative measures of Rudolphiella species.

Genus <i>Rudolphiella</i> Hosts Basin	R. szidati sp. n. R. lobosa syntypes Luciopimelodus pati "Pimelodus pati". Parana Parana	R. lobosa syntypes "Pimelodus pati" Parana	R. piracatinga syntypes Calophysus macrocephalus Amazon	R. piranabu syntypes Pinirampus pirinampu Amazon	R. myoides syntypes Pinirampus pirinampu Amazon	R. cf. lobosa (de Ch.+V. 1999) Megalonema platamun Parana
ø metascolex ø suckers % PC % ovary	1735-2970 395-595 15-32, m = 23 43-68, m = 53	1025-1350 205-245 16-22, m = 18 60-72, m = 65	340-360 *	580-680	290	925-1700 305-345 21-23, m = 22 54-62, m = 58
% genital pore testes number	20-46, m = 30 289-365, m = 325	21-32, m = 27 194-219, m = 206 8-13	40-50	+ 100 (80**)	100	18-20, III = 21 180-183 4-10
nb. uterine diverticies9-17 nb. of proglottis 22-37 Total length 16-27 Vagina position*** 77%	22-37 16-27 mm, m = 22 77% posterior	8-12 35 17-22 mm 53% posterior	15 6 mm	15 10 mm 81% posterior	15 11 mm 33% posterior	25-27 10 mm 28% posterior

* distorded in mounting according to Woodland (1935) ** according to Pavanelli & Machado dos Santos (1992)

*** position of vagina in comparison with cirrus pouch

supported. We observed that all *Rudolphiella* species shown some more synapomorphies: presence of glandular cells with granular inclusions arranged in a cross situated between the suckers and the tegument, eggs with elongated shell, embryophores bearing two polar projections, vitellines follicles cortical in two ventral bands situated sub-laterally, vagina anterior/posterior. Those synapomorphies shared by all *Rudolphiella* species emphasize the homogeneity of this group and enforced the Brooks' opinion on the monophyly of this genus.

Stewart (1986) stated that *Luciopimelodus pati*, *Pinirampus pirinampu* and *Calophysus macropterus* belong to a monophyletic group called the «Calophysus group», within the Pimelodidae, opinion confirmed by Lundberg *et al.* (1991) and more recently by de Pinna (1998). On the other hand, *Megalonema* shares some characteristics with the *Calophysus* group, and other groups closely related to Diplomystidae (primitive catfishes). In the light of our studies, the presence of *Rudolphiella* cf. *lobosa* (de Chambrier & Vaucher, 1999) from *Megalonema platanum* confirms the relationship of *M. platanum* with the *Calophysus* group.

In conclusion, the sole genus *Rudolphiella* is widespread among the genera of the «*Calophysus* group» we studied, including *Megalonema platanum*. It is, worthwhile to emphasize that it could be a likely case of coevolution between related species of hosts and their parasites.

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